

mal joints (6.1% vs. 2.2%), corresponding the highest percentage of cellular expression to the co-expression of CD90/CD271 (9.8% vs. 2.6%). Spontaneous repair tissue contained cells positive for CD44 (12.3%), CD90 (10.4%) and CD271 (9.9%) antigens, but the CD105 antigen was absent.

Conclusions: These results suggest that during the OA process an increment of MSC percentage occur in synovial membrane. Absent expression of the CD105 antigen in the OA cartilage repair indicates that cells expressing this marker may be necessary for effective repair processes in OA cartilage.

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EXPRESSION ANALYSIS OF mRNA DURING DIFFERENTIATION FROM MSCS TO MATURE CHONDROCYTES

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Purpose: Mesenchymal stem cells (MSCs) are considered as a potential cell therapy to treat a variety of rheumatic diseases. MSCs are widely distributed in the body, including bone marrow (BM). Chondrogenic differentiation process involves the differentiation of MSCs towards cartilage or bone. Cartilage tissue engineering uses efficient protocols that suppress differentiation towards hypertrophic chondrocytes, providing a useful in vitro model for studying the gene regulation of chondrogenesis and cartilage development. This model may provide new molecular targets for rheumatic diseases treatment. The aim of this study was investigate the transcriptional regulation of genes implicated in the different steps of chondrogenesis in normoxia versus hypoxia conditions.

Methods: MSCs, isolated from 5 BM were expanded in monolayer. CD105+ and CD105- cells were separated from total BM by MACS Technology (Miltenyi Biotec, Spain) and their phenotype were characterized by flow cytometry. The three populations of MSCs (total BM, CD105+ and CD105- BM) were subjected to chondrogenesis by means of high-density 3D aggregate culture in normoxia and hypoxia conditions. Chondrogenesis was studied at different intervals of time: 0, 2, 4, 7, 14, 21, and 28 days and differentiation was confirmed by histochemistry (hematoxylin-eosin, Masson's trichrome, alcian blue, safranin O), immunohistochemistry (types II and I collagens, and aggrecan) and quantitative real Time PCR (qPCR) techniques. For qPCR experiments RNAs were isolated using Trizol reagent (Invitrogen, Spain). Different primers were designed to study mRNA expression of different genes implicated in the different stages of chondrogenesis (MSCs, condensation & chondroprogenitors, chondrocytes and prehypertrophic chondrocytes). These primers included Sox2, Nanog, OCT3/4, CDH2, TNC, Sox9, Agg, Col I, Col II, Col X and RunX2.

Results: At the starting point of the experiments, the stem cell phenotype markers (SOX2, Nanog and OCT3/4) showed higher expression levels in the CD105+ population. At 14-28 days in hypoxia and at 21-28 days in normoxia the expression levels of Nanog and OCT3/4 showed to be higher in the total bone marrow and CD105- populations. Instead, the level of SOX2 at the same times of the experiment showed to be higher in the CD105+ population. These markers remained expressed until day 28, both in normoxia and in hypoxia, indicating that not all the stem cells were differentiated towards chondrocytes but some of them

remained in a state of indifferentiation. With regard to cartilage specific markers, the CD105- population showed increased levels of SOX9 and AGG mRNA than CD105 + and total bone marrow ones, however the latter showed the highest expression levels of Col II followed by CD105- and CD105 + populations. In this way, the overexpression of Col II in hypoxia was accentuated between 7 and 21 days while in normoxia took place from 14 to 28 days.

Conclusions: Hypoxia accelerates the chondrogenic differentiation process from bone marrow-derived adult mesenchymal stem cells to mature chondrocytes. Isolation of CD105 + and CD105- populations shows no improvement in chondrogenesis with regard to the total bone marrow population. In addition, while the CD105+ population seems to differentiate in the experimental conditions, the CD105- one shows to remain in an indifferentiated state, maintaining an important expression of SOX2, NANOG and OCT3/4 genes.

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AN ORGAN CULTURE MODEL TO STUDY BIOLOGICAL REPAIR OF THE DEGENERATE INTERVERTEBRAL DISC

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The current surgical procedures used to treat the degenerate intervertebral disc (IVD) are inadequate. Vertebral fusion can frequently reduce back pain, but does not preserve motion segmental mobility and thus may lead to subsequent degeneration in adjacent motion segments. In contrast, artificial disc prostheses preserve mobility, but in the long-term may result in serious complications due to wear, loosening, subsidence or mechanical failure. Biological repair of the degenerate IVD could provide a means of overcoming these problems. In vitro organ culture models using large IVDs have recently been described for this purpose, but they have suffered from the inability to achieve long-term NP cell viability, due to impaired nutrient supply because of endplate calcification and the postmortem formation of blood clots blocking the trans-endplate nutrition path.

Purpose: To develop a bovine coccygeal IVD organ culture model of disc degeneration with long-term cell survival, in which induced biological repair can be studied.

Methods: IVDs were isolated from bovine tails by parallel cuts through the adjacent vertebral bodies, and remaining bone and most of the cartilage endplates were removed using a high-speed bone burr. The explants were maintained in culture in DMEM, supplemented with 5% FBS and antibiotics, with medium changed twice weekly. Degeneration was induced by injecting trypsin into the center of the NP. Cell viability was monitored in a 1 mm slice through the center of the disc, using a live/dead fluorescence assay (Live/Dead®, Invitrogen) and confocal microscopy. Tissue morphology was examined by histology. Annulus fibrosus (AF) and nucleus pulposus (NP) were extracted using 15 volumes 4M GuHCl containing protease inhibitors, and changes in the abundance and degradation of chondroadherin and fibromodulin were analysed by immunoblotting. GAG content was measured using the DMMB assay.

Results: Intact bovine discs maintained in free swelling culture with no external load applied demonstrated cell viability for up to 6 weeks. The amount of GAG retained in the tissue decreased slowly during the culture period and cell clustering reminiscent of cartilage and disc degeneration was found. As it is not practical to wait for 4-6 weeks before repair potential of various treatments